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# Ethanol production from non-detoxified whole slurry of sulfite-pretreated empty fruit bunches at a low cellulase loading ☆

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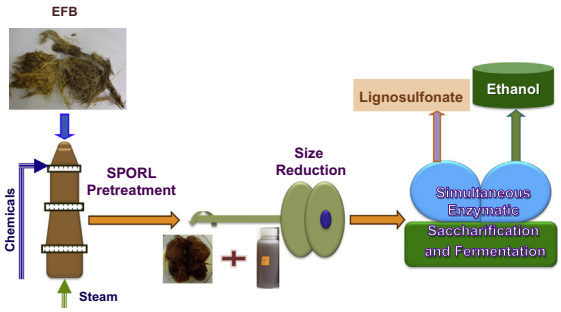
## HIGHLIGHTS

- High yield of 217 L/tonne ethanol production from EFB pretreated by SPORL.
- Enzymatic hydrolysis and fermentation of non-detoxified pretreated whole slurry.
- Low cellulase loading of 15 FPU/g glucan.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Sulfite pretreatment to overcome the recalcitrance of lignocelluloses (SPORL) was applied to an empty fruit bunches (EFB) for ethanol production. SPORL facilitated delignification through lignin sulfonation and dissolution of xylan to result in a highly digestible substrate. The pretreated whole slurry was enzymatically saccharified at a solids loading of 18% using a relatively low cellulase loading of 15 FPU/g glucan and simultaneously fermented without detoxification using *Saccharomyces cerevisiae* of YRH400. An ethanol yield of 217 L/tonne EFB was achieved at titer of 32 g/L. Compared with literature studies, SPORL produced high ethanol yield and titer with much lower cellulase loading without detoxification.

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## 1. Introduction

Empty fruit bunches (EFB) are lignocellulosic residues from the palm oil industry. In Malaysia alone, the annual production of approximately 93 million tonnes of fresh fruit bunches (FFB) resulted in 44 million tonnes of oil palm residue solids, of that, 24 million tonnes are EFB (Chiew and Shimada, 2013). EFB has high moisture content when wet and is bulky when dried, which is not favorable for transportation. Currently, EFB is often left to rot at mills or plantations. Using EFB to produce ethanol could be a way to use this bioresource with lower environmental impact than for the production of pulp, paper or medium density fibers (MDF) (Chiew and Shimada, 2013). Unfortunately, EFB is highly

☆ This work was conducted when Cheng and Leu were visiting scientists at the USDA Forest Products Laboratory and on official government time of Zhu.

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recalcitrant to enzymatic deconstruction for saccharification and fermentation. Various pretreatment methods have been explored for biochemical conversion of EFB. However, few studies produced good sugar yield without very high cellulase loadings in enzymatic hydrolysis (Choi et al., 2013; Jung et al., 2011, 2013b; Shamsudin et al., 2012; Tan et al., 2013). To illustrate the difficulties using EFB for ethanol production, we have summarized results from several recent studies in Table 1. Some studies discarded the hemicellulose sugar stream and fermented only the washed solids, not the pretreated whole slurry to avoid the difficulties due to fermentation inhibitors. See for example (Choi et al., 2013; Tan et al., 2013). Others only fermented the pretreatment hemicellulosic sugar stream (Lau et al., 2010) without considering the effectiveness of the pretreatment for achieving high enzymatic saccharification of the cellulose fraction; therefore actual ethanol yields from the whole EFB residue are not available. In one study that used an alkaline pretreatment and a detoxification step the yield was only 67 L/tonne (Piarpuzán et al., 2011). In another study using a dilute acid pretreatment followed by acid hydrolysis and fermentation, the yield was 112 L/tonne (Millati et al., 2011). A recent study reported an ethanol yield of 172 L/tonne or 61.3% of theoretical based on EFB glucan content when dilute maleic acid pretreatment was used in combination with a high cellulase loading of 30 FPU/glucan (Jung et al., 2013b).

In most of these cases, the main reason for low ethanol yields was inefficient pretreatment that resulted in poor substrate digestibility. A severe pretreatment can improve enzymatic saccharification efficiency but it can also increase sugar degradation, which results in the formation of fermentation inhibitors that reduce ethanol yield. For example, ethanol yield was reduced to 148 L/tonne when sulfuric acid was used (Jung et al., 2013a) instead of maleic acid when both pretreatments were conducted under the same conditions (Jung et al., 2013b) (Table 1). The yield improved by 53% to 246 L/tonne when sulfuric acid pretreated whole slurry was detoxified using activated carbon (Jung et al., 2013a). Thus, harsh pretreatment reduces yield by inhibiting fermentation, and by converting the substrate into useless toxic byproducts. To avoid high concentration of fermentation inhibitors all of the studies cited above used whole EFB slurry at solids contents of 10% or less, which resulted in low ethanol titer of less than 20 g/L (Table 1). Ethanol yields were also reduced by inefficient utilization of xylose by the yeasts employed. Therefore, yield in terms of percent of theoretical was often reported based on EFB glucan content. However, the yield was only 130 L/tonne when using a xylose fermenting strain after EFB pretreated through mechanical milling (Yano et al., 2009).

In the study reported here, we improved enzymatic saccharification efficiency and therefore ethanol production by applying the sulfite pretreatment to overcome the recalcitrance of lignocelluloses (SPORL). In our previous studies the SPORL pretreatment has shown robust performance in removing the recalcitrance of softwoods (Zhou et al., 2013b; Zhu et al., 2009a) and softwood forest residue (Leu et al., 2013). SPORL was developed based on sulfite pulping that has proven commercial scalability. Partial delignification through sulfonation produces lignosulfonate (LS) that can be marketed along with ethanol. Furthermore, LS has low affinity to cellulase and acts as surfactant to enhance enzymatic saccharification (Wang et al., 2013; Zhou et al., 2013a). Therefore it is possible to eliminate the washing of pretreated solids, which reduces water usage and facilitates direct enzymatic saccharification and fermentation of the pretreated whole slurry. These are significant advantages compared with alkaline, dilute acid and other pretreatments.

A different research group recently reported using SPORL to pretreat EFB (Tan et al., 2013). However, their study used a pretreatment temperature of 180 °C, which converted significant amounts of hemicelluloses into furan and prevented fermentation of the whole slurry. Our previous studies had shown that sugar degradation to furan has a higher activation energy than hemicellulose dissolution (Zhang et al., 2014a), therefore by using a lower temperature with longer pretreatment time while maintaining the same pretreatment severity can reduce furan formation without reducing hemicellulose dissolution. Such an approach could increase enzymatic saccharification while avoiding the formation of toxic products. The present study showed that a lower temperature SPORL pretreatment combined with a low cellulase loading could attain a high ethanol yield from EFB without detoxification and without washing.

## 2. Methods

### 2.1. Materials

Oil palm EFB was supplied by the Teck Guan Group, Sabah, Malaysia. The EFB was derived from fresh empty fruit bunches

**Table 1**
Summary of literature studies on ethanol production from EFB along with comparison with the present study.

| Source | Year | Pretreatment | Cellulase (FPU/g glucan) | Fermentation | Titer (g/L) | Yield (L/tonne;% theoretical [a]) |
|---|---|---|---|---|---|---|
| *Whole slurry* | | | | | | |
| Piarpuzán et al. (2011) | 2011 | Soaked in 2% v/v NaOH at 30 °C for 4 h, then autoclave at 121 °C for 6 min, 10:1 liquor to EFB | 107 | SHF of whole slurry @ 10% with $(NH_4)_2HPO_4$; $MgSO_4$; $NaH_2PO_4$; $CO(NH_2)_2$; $FeCl_3$ Overliming + Sulfite for detoxification | <5 | 67; 19.8 |
| Millati et al. (2011) | 2011 | 0.8% $H_2SO_4$ at 170 °C for 15 min then at 210 °C for 5 min, 10:1 liquor to EFB | Two stage acid hydrolysis | Fermentation of 1st stage hydrolysates for C5 and 2nd stage for C6 sugar | 2.4 | 112; 43.4 |
| Jung et al. (2013b) | 2013 | 1.0 w/v% maleic acid at 190 °C for 3 min, 10:1 liquor to EFB | 30 | SSF of whole slurry @ 10% No detoxification | 10.4 | 172; 61.3 |
| Jung et al. (2013a) | 2013 | 1.0 w/v% $H_2SO_4$ at 190 °C for 3 min, 10:1 liquor to EFB | 30 | SSF of whole slurry @ 6% — No detoxification | <10 | 148; 52.5 |
| | | | | SSF of whole slurry @ 6% — Activated carbon for detoxification | <15 | 246; 87.5 |
| Present study | | Sodium bisulfite 7% of EFB pH = 1.6–2.0 at 165 °C for 75 min, 4:1 liquor to EFB | **15** | SSF of whole slurry @ 18%, xylose use No detoxification | **32** | **217**; **99.0**[b] |
| *Washed solids only* | | | | | | |
| Tan et al. (2013) | 2013 | Sodium bisulfite 8% of EFB pH = 2.3 at 180 °C for 30 min, 4:1 liquor to EFB | 40 | SSF at 18% washed solids | 52.0 | 243; 91.7 |
| Choi et al. (2013) | 2013 | Soaking in 3% NaOH the steamed at 160 °C for 11 min 20 s | 40 | SSF at 10% washed solids | 25.3 | 228; 80.7 |

[a] Based on EFB glucan only.
[b] near 100% due to xylose utilization.

by steaming at 134 °C (3 bar) for 90 min, shredded while still hot, then dried in an oven at 60 °C for 2 days. The dried sample was stored in freezer at −20 °C until shipped to Madison, WI. The steaming process can remove some hemicelluloses similar to steam pretreatment and drying of pretreated lignocelluloses can cause the collapse of fiber pores to reduce cellulose accessibility to cellulase (Luo and Zhu, 2011; Wang et al., 2012). As a result, the received EFB was very recalcitrant.

Commercial cellulase cocktails of CTec2 and CTec3 were kindly provided by Novozymes North America (Franklinton, NC). The measured cellulase activities were 147 and 217 filter paper unit (FPU)/mL for CTec2 and CTec3, respectively. *Saccharomyces cerevisiae* of YRH400 is an engineered yeast strain for xylose fermentation (Hector et al., 2011) and was kindly provided by National Center for Agricultural Utilization Research of USDA Agriculture Research Service, Peoria, IL.

### 2.2. SPORL pretreatment of EFB

For convenience in laboratory study, the dilute sulfite solution was prepared using sodium bisulfite and sulfuric acid, rather than bubbling $SO_2$ into a hydroxide solution as practiced in sulfite pulp mills. Two levels of sodium bisulfite charge on od weight EFB of 4% and 7% were used. At each sulfite loading, sulfuric acid loading on od EFB weight were varied from 0.4% to 2.5%. A dilute acid pretreatment (bisulfite loading = 0) at acid charge of 2.5% was also conducted. As received EFB fibrous material, in the form of bunch of large fibers, without size reduction of 75 g in od weight was directly mixed with the prepared dilute sulfite solution for pretreatment. All pretreatments were conducted at 165 °C for 75 min at liquor to EFB in oven dry weight (od) ratio of 4 to 1 using 1 L bomb reactors. Three 1 L bomb reactors were mounted in a 23 L wood pulping digester in an autoclave configuration as described previously (Luo et al., 2010). The wood pulping digester was heated by a steam jacket and rotated at 2 rpm for mixing. The heating time to 165 °C was approximately 8 min.

At the end of pretreatment, the digester was cooled down using tap water. After the pretreatment, 2 mL spent liquor was collected for chemical analysis. The rest of the solids and liquor were directly milled together to produce pretreated whole slurry using a 12-in disk refiner (Andritz Sprout-Bauer Atmospheric Refiner, Springfield, OH) for fermentation. The disk plate pattern of D2-B505 was used with a disk plat gap of 1 mm, an order of magnitude greater than that used for wood pulping. Because of the small amount of material (<75 g) after pretreatment, energy consumption for milling cannot be accurately measured. The large disk plate gap and the loosened biomass structure through SPORL pretreatment ensured a very low mechanical milling energy consumption of approximately 50 wh/kg untreated EFB based on our previous studies using pretreated wood (Zhu et al., 2009b, 2010).

### 2.3. Enzymatic hydrolysis of washed pretreated solids

Enzymatic hydrolysis was conducted using washed solids to evaluate the effectiveness of SPORL pretreatment for saccharification. Approximately 100 g of wet refined whole slurry was washed thoroughly using deionized water. The washed solids were analyzed for chemical composition. Hydrolysis experiments were conducted at solids loading of 2% (w/v) in 100 mL flasks on a shaker (Thermo Fisher Scientific, Model 4450, Waltham, MA) at 50 °C and 200 rpm. Sodium acetate buffer of pH 5.5 was used. The elevated pH of 5.5 higher than commonly used pH of 4.8–5.0 was found to enhance enzymatic saccharification (Lan et al., 2013; Lou et al., 2013). CTec2 loading was 15 FPU/g glucan. Hydrolysates were taken at predetermined intervals and then centrifuged. The supernatant was analyzed for glucose by a biochemistry analyzer (YSI 2700S, YSI Inc., Yellow Springs, OH, USA).

### 2.4. Quasi-simultaneous saccharification and fermentation (SSF) of pretreated whole slurry

Each pretreated and refined EFB whole slurry sample was neutralized to pH 5.5 using lime, and directly used for enzymatic saccharification and fermentation at 18% total solids using *S. cerevisiae* YRH400 without detoxification. Cellulase CTec3 loading was 15 FPU/g glucan. Citric acid/sodium citrate buffer (50 mM) of pH 6.5 was added into the pH adjusted cellulase and whole slurry mixture in 250 mL Erlenmeyer flasks. Each buffered mixture was first liquefied on a shaker (Thermo Fisher Scientific, Model 4450, Waltham, MA) at 50 °C and 200 rpm. The liquefaction time varied from sample to sample due to variations in pretreatment. Typical liquefaction time was between 8 and 12 h due to lack of shear mixing. The mixture was then cooled down to 35 °C and the shaker speed was reduced to 90 rpm and inoculated with yeast seed. The initial optical density of the yeast for all fermentation experiments was controlled at OD600 = 5. No additional nutrients were applied during fermentation. Samples were taken at predetermined intervals (0, 3, 7, 24, 48, 72, 96, 120, 144, 150 h), and then centrifuged. The composition of the supernatants was analyzed using a HPLC as described previously (Zhou et al., 2013b).

### 2.5. Analytical methods

The untreated EFB was first extracted by hot water and ethanol following a procedure of the U.S. National Renewable Energy Laboratory (Sluiter et al., 2008) using a Soxhlet extractor. The percentage of biomass extracted was determined from the oven dry weight loss of the biomass through extraction.

A two stage acid hydrolysis procedure was used for analyze the chemical compositions of the extracted but untreated and pretreated EFB solids using anion exchange chromatography as described previously (Luo et al., 2010). Klason lignin was measured gravimetrically. As a result, the extractives remained in the pretreated solids as revealed in a previous study (Tan et al., 2013) may affect the lignin measurements. Chemical compositions of the pretreated spent liquors and fermentation broths were analyzed using a Dionex HPLC system (Ultimate 3000, Thermo Scientific) equipped with BioRad Aminex HPX-87P and 87H columns as described previously (Zhou et al., 2013b).

## 3. Results and discussion

As noted above, steaming of fresh fruit empty bunches followed by drying is a common practice to produce EFB for easing shipping, logistics, and storage. This reduced the hemicellulose content, and more critically, by collapsing the structure of the glucan, it increased recalcitrance. In a commercial process, using wet EFB, it should be possible to attain higher hemicellulose and glucan yields. The process descried here used a strain of *S. cerevisiae* engineered for xylose fermentation, which could benefit from higher xylan content and enzymatic glucan yields.

### 3.1. Effect of pretreatment on dissolution of hemicelluloses

Hemicellulose removal was previously found to be the dominant factor that can improve the enzymatic digestibilities of plant biomass with low lignin contents (Leu and Zhu, 2013; Moxley et al., 2012; Zhang et al., 2013; Zhu et al., 2012). Both acid and sulfite loading affect hemicellulose dissolution. Reducing the pH by increasing acid loading improved xylan removal (Fig. 1a). Approximately 85% of xylan could be dissolved by pretreatment with an

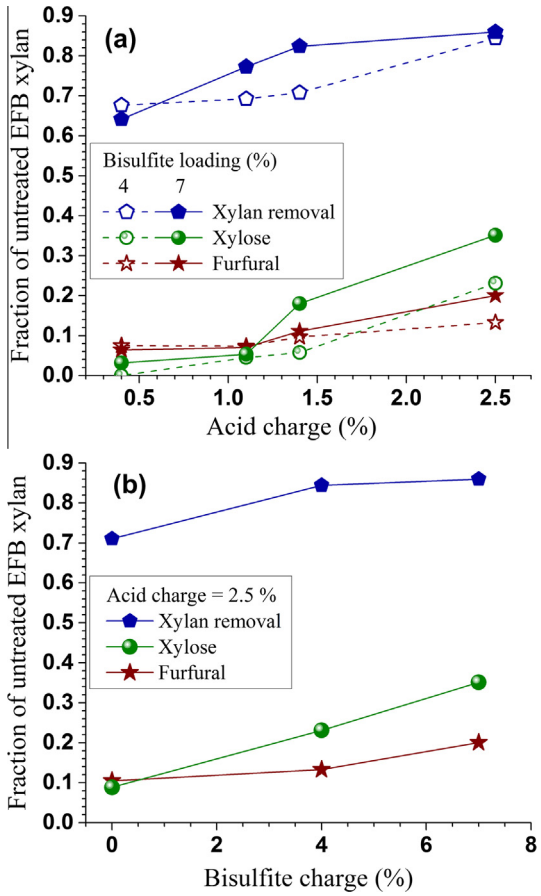


**Fig. 1.** Effect of pretreatment chemical loadings on xylan removal, xylose and furfural productions. (a) Acid charge on oven dry EFB (%) and (b) sodium bisulfite charge on oven dry EFB.

acid charge of 2.5%. Increasing sulfite loading while keeping the acid charge constant also increased xylan dissolution, as sulfite is known to be capable of depolymerizing hemicelluloses. Part of the dissolved xylan was hydrolyzed to xylose, and some of the resulting xylose was degraded into furfural. The data shown in Fig. 1a shows a significant discrepancy between the fraction of xylan dissolution and the total yield of xylose and furfural, suggesting that a significant amount of xylan was only partially hydrolyzed to xylooligomers. The xylooligomers content was approximately 30% of EFB xylan at a sulfite loading of 7% and an acid charge of 2.5%.

To further evaluate the effect of sulfite loading on hemicellulose removal, the results from a dilute acid pretreatment were also plotted (Fig. 1b). Xylan dissolution increased almost linearly with sulfite loading at acid charge of 2.5%, which resulted in a linear increase in xylose yield over the range of sulfite concentrations studied. Dilute acid pretreatment removed only 70% of EFB xylan. Furfural formation was also increased with sulfite loading, however, the amount of increase is lower than the amount of xylose produced, suggesting increase sulfite loading favors xylan hydrolysis over xylose degradation.

### 3.2. Effect of pretreatment on enzymatic digestibility

Sulfite application is critical to remove the recalcitrance of EFB through delignification and hemicellulose dissolution, as we demonstrated previously (Zhang et al., 2014a,b). However, we were not able to accurately quantify lignin removal using a gravimetric determination of Klason lignin due to the presence of extractives and ash. A previous study indicated that significant amounts of extractives remained after SPORL pretreatment (Tan et al., 2013). Furthermore, carbohydrate can be degraded to "Pseudo lignin" under acidic conditions (Sannigrahi et al., 2011). When compared to dilute acid pretreatment under the same acid loading and pretreatment temperature and duration, sulfite addition improved enzymatic saccharification efficiency of pretreated EFB (Fig. 2a). Saccharification efficiency or SED is defined as the percent of substrate glucan enzymatically saccharified to glucose. A higher SED is primarily due to increased xylan removal and secondarily due to delignification, as discussed previously, which agree with other reported studies using sulfite pretreatment (Leu et al., 2013; Mendes et al., 2011; Zhang et al., 2014a) A sulfite loading of 7% (Fig. 2a) as well as reduced pH from higher acid loading improved enzymatic saccharification efficiency (Fig. 2b), again due to increased hemicellulose dissolution and delignification (Fig. 1a).

To reveal the role of xylan dissolution on enhancing enzymatic saccharification of pretreated EFB, the 72-h SED hydrolysis data of all 9 pretreatments were plotted against xylan removal. The data fitted well to a linear relationship for pretreatments conducted at the same bisulfite loading (Fig. 3), suggesting xylan removal is the dominant factor affecting cellulose saccharification. However, a smaller slope, i.e., less dependence on xylan removal, was observed for pretreatments conducted at bisulfite loading 7% than those at 4%, suggesting increased delignification at an increased bisulfite dosage of 7% reduced the effect of xylan removal on SED. In other words, delignification can compensate low xylan removal to achieve high SED. When EFB was exposed to dilute acid pretreatment alone (sulfite = 0), no lignin is sulfonated and only the acid soluble lignin fraction (a minimal amount) is removed. The SED of this dilute acid pretreated sample is significantly below the two linear regression lines with delignification at the same xylan removal. This indicates, a certain amount of lignin removal

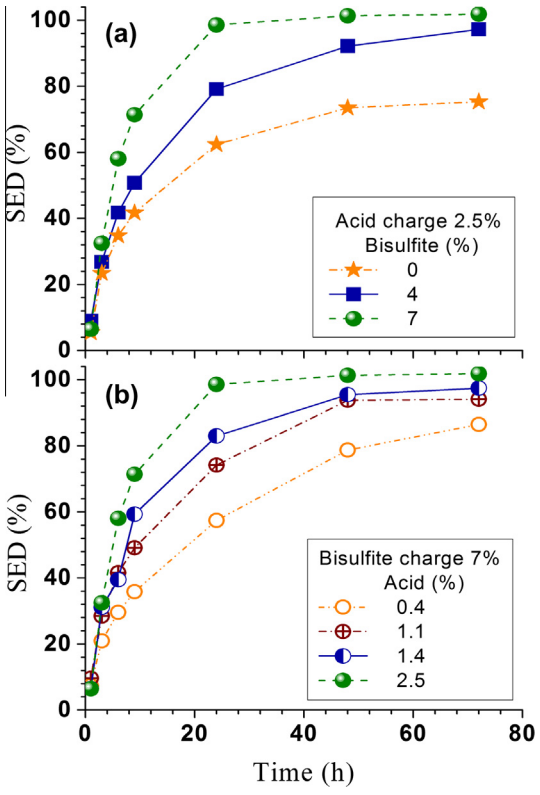


**Fig. 2.** Time-dependent substrate enzymatic digestibility (SED) under various pretreatment chemical loadings. (a) Sodium bisulfite variation and (b) acid variation.

through lignin sulfonation is necessary to improve substrate digestibility. However, the influence of increased delignification at high bisulfite loading of 7% was not reflected in SED at high xylan removal of approximately 85% (Fig. 3). This confirmed that significant delignification is not necessary for biomass with low lignin content such as EFB (Leu and Zhu, 2013).

### 3.3. Ethanol production and sugar consumption

Selected pretreated EFB whole slurry samples were used for saccharification and fermentation at total solids of 18%. These samples all have relatively good saccharification efficiency, i.e., SED at 72 h approximately of 85% or higher. As shown in Fig. 4, terminal ethanol of over 30 g/L was achieved for the two samples pretreated with sulfite loading of 7% and acid charge of 1.4 (W-A14B7) and 2.5% (W-A25B7). We used the form of AxxBx to label the samples based on Table 2. "W" stands for whole slurry. The pretreatments used sulfite charge of 4% produce low ethanol titer. Examining the SED data indicates that the high saccharification efficiencies of W-A14B7 and W-A25B7 facilitated fermentation, despite these two samples had more furfural than the corresponding sample derived from bisulfite loading of 4% (Fig. 1a and b). While the saccharification performance of W-A25B4 is similar to that of W-A14B7 (Fig. 2), the significant amount of oil that was observed in W-A25B4 may have inhibited fermentation in addition to the higher amount of furfural in W-A25B4 than in W-A14B7 (Fig. 1a). This suggests that oil may have inhibitive effect on yeast fermentation. It is known that extractives can form "oily stickies" under acidic conditions in sulfite pulping. The Run A25R4 represents the most acidic pretreatment.

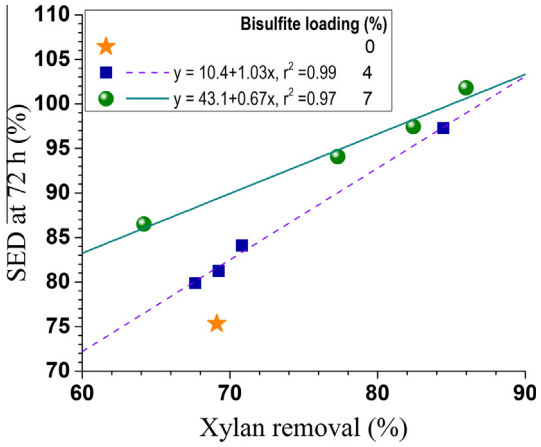


**Fig. 3.** Correlation between xylan removal and terminal substrate enzymatic digestibility (SED) at 72 h.

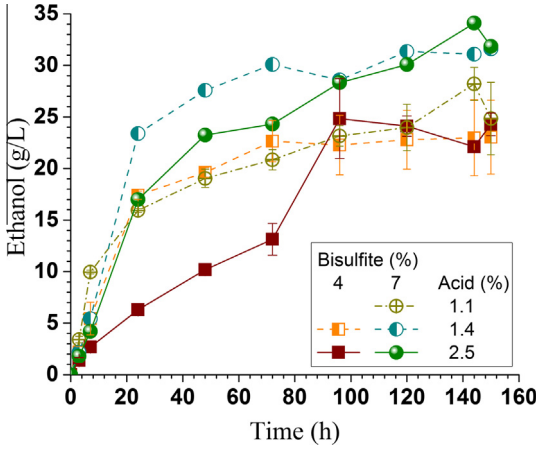


**Fig. 4.** Time-dependent ethanol production for five SPORL-pretreated EFB.

Glucose consumption was rapid for most fermentation runs except for the two replicate runs using W-A25B4 that contained oil (Fig. 5a). Xylose consumption was limited as shown in Fig. 5b even though YRH-400 was engineered for xylose fermentation. This can be attributed to the limited effectiveness of the strain for xylose fermentation as well as the amount inhibitors present (Zhou et al., 2014). Furan metabolization was rapid (Fig. 5c) except for the run using W-A25B4 that is most acidic and has "oily stickies".

### 3.4. Comparisons with literature studies

Despite many efforts to use EFB for biofuel production, few studies have reported fermentation using the pretreated whole slurry without detoxification (Table 1). The approach described here is capable of producing 217 L ethanol/tonne EFB at 32 g/L without detoxification by directly conducting enzymatic saccharification and fermentation of the pretreated whole slurry using a relatively low cellulase dosage of only 15 FPU/g glucan. Most literature studies produced significantly lower ethanol yields of less than 200 L/tonne when even though high cellulase loadings were used (Table 1). Only when the whole slurry was detoxified using activated carbon, could an ethanol yield of 248 L/tonne be achieved at a high cellulase loading of 30 FPU/g glucan.

It was reported that discarding the pretreatment spent liquor that contains the hemicellulose sugar stream, using the washed pretreated EFB produced high ethanol yield at a high cellulase loading of 40 FPU/g glucan (Table 1). In view of the low cellulase loading of 15 FPU/g glucan in the present study, we believe that SPORL has great potential for EFB bioconversion. Further improve-

**Table 2**
Chemical compositions of the untreated and SPORL pretreated washed EFB.

| Sample [a] | Chemical loading | | K. Lignin | Glucan | Xylan | Mannan | Arabinan | Galactan | Solids yield (%) |
|---|---|---|---|---|---|---|---|---|---|
| | Acid (mL/L) | Bisulfite on wood (%) | | | | | | | |
| Untreated | Extractives = 17.3 ± 0.4 | | 17.1 ± 0.9 | 30.5 ± 0.6 | 16.0 ± 0.4 | 0.7 ± 0.0 | 1.5 ± 0.1 | 0.5 ± 0.1 | 100 |
| A04B4 | 4 | 4 | 32.6 ± 0.8 | 46.9 ± 0.7 | 8.4 ± 0.6 | 1.1 ± 0.4 | Nd | 0.9 ± 1.0 | 61.73 |
| A11B4 | 11 | 4 | 31.1 ± 0.2 | 47.9 ± 1.3 | 7.9 ± 0.4 | 1.2 ± 0.5 | Nd | 0.2 | 62.07 |
| A14B4 | 14 | 4 | 33.9 ± 0.3 | 47.3 ± 0.4 | 7.3 ± 0.2 | 1.2 ± 0.5 | Nd | 0.2 | 63.68 |
| A25B4 | 25 | 4 | 40.2 ± 0.4 | 45.5 ± 0.1 | 4.4 ± 0.4 | 0.2 | Nd | 0.1 | 56.56 |
| A25B0 | 25 | 0 | 38.2 ± 0.0 | 44.8 ± 1.8 | 7.1 ± 0.1 | 1.0 ± 0.6 | Nd | 0.1 | 65.35 |
| A04B7 | 4 | 7 | 26.8 ± 1.3 | 48.8 ± 0.0 | 9.4 ± 1.3 | 1.1 ± 1.0 | Nd | 0.2 | 61.16 |
| A11B7 | 11 | 7 | 32.5 ± 0.2 | 46.6 ± 0.6 | 6.3 ± 0.3 | 1.0 ± 0.4 | Nd | 0.2 | 58.05 |
| A14B7 | 14 | 7 | 34.3 ± 0.5 | 48.2 ± 0.0 | 5.0 ± 0.0 | 0.8 ± 0.3 | Nd | Nd | 56.59 |
| A25B7 | 25 | 7 | 37.1 ± 0.0 | 49.1 ± 1.5 | 4.0 ± 0.6 | 0.6 ± 0.3 | Nd | Nd | 55.81 |

[a] A number is sulfuric acid in mL/L and B number is sodium bisulfite charge on wood in oven dry base.

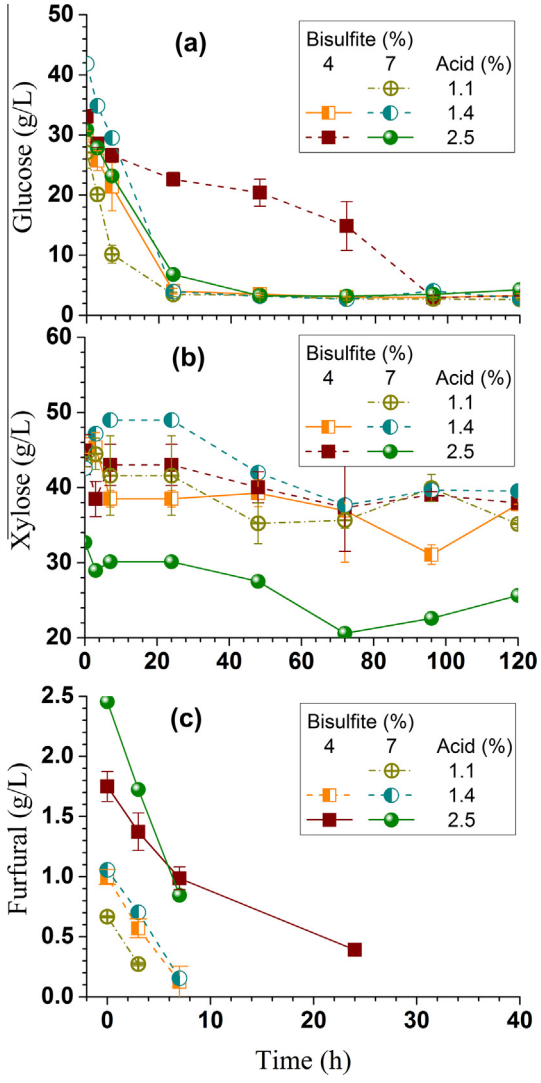


**Fig. 5.** Time-dependent sugar consumption and furan metabolization during fermentation of five SPORL-pretreated EFB. (a) Glucose, (b) xylose and (c) furfural.

ment can be made in terms of ethanol yield and titer based on our studies using softwoods (Zhou et al., 2013b).

## 4. Conclusions

This study demonstrates that SPORL can effectively reduce the strong recalcitrance of empty fruit bunches (EFB) to produce an ethanol yield of 217 L/tonne at 32 g/L without detoxification at relatively low cellulase loading of 15 FPU/g glucan. Compared with all prior studies using different pretreatments, SPORL demonstrated significant advantages by eliminating washing of pretreated solids, and reducing cellulase loading due to lignin sulfonation. Further optimization of SPORL pretreatment could improve ethanol yield at high titer, and we would expect the process to perform better using wet EFB as would be found prior to steaming and drying.

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